



Plant Research Department annual report 2002

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Publication date:
2003

Document Version
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

Citation (APA):
Kossmann, J., Jakobsen, I., Nielsen, K. K., Pilegaard, K., Rasmussen, S. K., & Thordal-Christensen, H. (Eds.) (2003). *Plant Research Department annual report 2002*. Denmark. Forskningscenter Risoe. Risoe-R No. 1398(EN)

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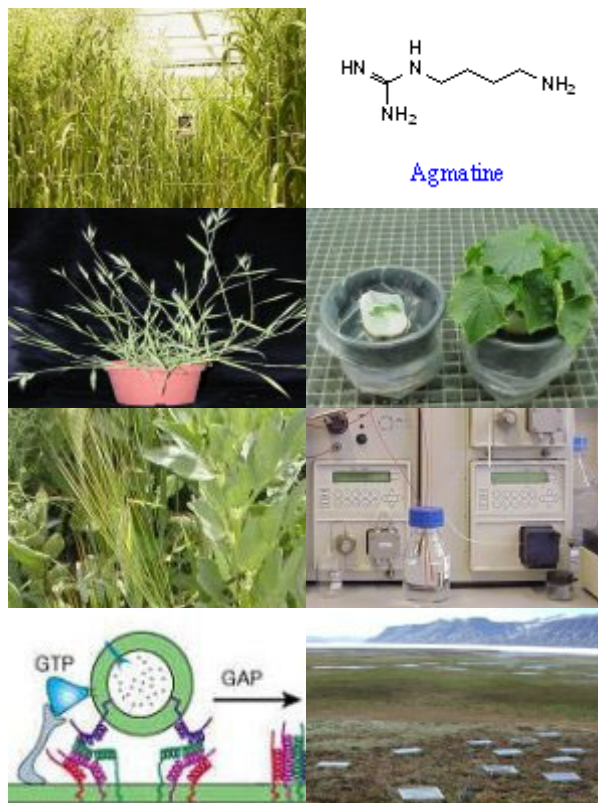
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Annual Report 2002



Plant Research Department



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Plant Research Department

Abstract

The Plant Research Department at Risø National Laboratory has the unique opportunity to be the only life science department located in an environment that is largely dominated by physicists. In 2002 increasing numbers of projects have been initiated that establish interdisciplinary research in order to take optimal advantage of the different fields of expertise that are established at Risø National Laboratory. These activities are mainly related to develop novel post-genomic tools to assign function to genes, which are widely applicable in the life sciences. The Plant Research Department applies these and state-of-the-art technologies to increase knowledge to develop crops with improved agronomic traits and to engineer high-value compound containing plants, which are, in addition, able to meet the growth conditions of the future environment with elevated temperatures and increasing carbondioxide and ozone concentrations in the atmosphere. Finally, activities are increasing to establish systems that optimize the production of energy from biomass in order to promote sustainability in industrial societies.

The department is divided into five research programmes that are linked through their individual [expertise](#) delivered to the rest of the department. Three programmes are engaged in improving the agronomic performance of plants. Genetic and molecular genetic tools are developed to enhance the nutrient efficiency of plants, to strengthen the

withstanding of plants to fungal attack, or to adapt the flowering time to the optimal use of crops. One programme is devoted to improve the market value of plant products. Plants with enhanced nutritional value or that contain novel renewable resources are designed to add value to the European Agro-Industries. A fifth programme ultimately is studying the effects of the future climate on plant growth, and the performance of newly designed crops and their interaction with the environment.

Diverse activities in the area of Functional Genomics integrate the department within itself, within the research environment at Risø National Laboratory, and finally within the Plant Science environment in Denmark and Europe. Each programme covers special expertises in the fields of genome, transcriptome, proteome, and metabolome analysis, which are delivered throughout the department and to other collaborators. It is unique to The Plant Research Department that these activities are supplemented with a broad expertise in environmental analysis, allowing the interpretation of large biological data sets in the context of factors affecting plant growth. In order to take optimal advantage of the acquisition of such massive data sets the development of unique bioinformatic tools is required, which is currently initiated through a pilot study in interaction with theoretical biophysicists.

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Risø National Laboratory

Mission

Risø's mission is to promote an innovative and environmentally sustainable technological development within the areas of energy, industrial technology and bioproduction through research, education, innovation and advisory services.

Vision

Risø's research shall **extend the boundaries** for the understanding of nature's processes and interactions right down to the molecular nanoscale.

The results obtained shall **set new trends** for the development of sustainable technologies within the fields of energy, industrial technology and biotechnology.

The efforts made shall **benefit** Danish society and lead to the development of new multi-billion industries.

Risø's activities in 2001 are reported in the following publications: Risø Annual Report (available in Danish and English), Risø's Annual Performance Report (Danish) and the annual progress reports of the research departments (English). All publications and further information can be obtained from risoe.dk. Printed publications are available from the Information Service Department, tel.: +45 4677 4004, e-mail: risoe@risoe.dk, fax: +45 4677 4013.

DLF-Risø Biotechnology Programme

Objectives

The biotechnology consortium between the Danish seed company DLF-TRIFOLIUM A/S and Risø National Laboratory, Plant Research Department, is conducting a research programme focused on developing technologies for the control of flowering and improving quality traits in ryegrass, including abiotic stress tolerance, digestibility, and nutritional value.

Research fields

- Identification of key genes responsible for the switch from vegetative growth to flowering
- Gene activation systems allowing control of flowering and seed production
- Test of isolated genes in transgenic grasses
- Abiotic stress tolerance
- Digestibility

- Nutritional value (fructans)
- Establishment of a monocot model transformation system
- Transposon exon trapping lines in *Arabidopsis*
- Identification of novel specific promoters
- Identification of mutations affecting the response to vernalisation

Selected report

- [Improving the nutritional value of pasture grass](#)
- [Expression of a 434:VP16 chimeric activator leads to high-level activation of gene expression in Arabidopsis](#)

Contact:

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Plant Environment Interactions Programme

Objectives

The aim of the programme is to study the structure, function, processes, and dynamics of agro- and semi-natural ecosystems, and the biological interactions between crops and wild plants. The goal is to predict the function of plant ecosystems in a changing environment and to assess the ecological risks of introducing genetically modified plant in the agricultural systems.

Research fields

- Soil-plant-atmosphere interactions in relation to air pollution, global change and other environmental aspects, and ecophysiology related to nutrient cycling and stress.
- Functioning of terrestrial ecosystems and impacts of global change.
- Plant fitness, competition, and environment
- Genetic resources for crops of the future.
- Ecosystem modelling. Developing and improving nutrient cycling models and ecophysiological models.
- Carbon sequestration in forest and grassland ecosystems.
- Emissions of non-CO₂ greenhouse gases and relationships with soil nutrient dynamics in organic and conventional grasslands and forest ecosystems.
- Gene flow between crops and wild relatives.
- Risk analysis of genetically modified crops.
- Development of sensors and sensor systems for site specific fertilisation. Improving the nitrogen utilisation by Precision farming.

Selected reports

- [Responses to elevated CO₂ and drought-stress in old and new varieties of oats, *Avena sativa*.](#)
- [Effects of UV-B radiation on the high arctic vegetation in Northeast Greenland](#)

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Plant Nutrition Programme

Objectives

The overall goal is to improve sustainability of crop production via the development of technologies for enhancing the efficiency of plant nutrient uptake. The achievement of this goal is based on a strategy combining functional genomics and nanobiotechnology with studies of plant physiology. The functional mapping of plant nutrient uptake has its focus on mycorrhizal fungi and root hairs and makes use of plant mutants, isotope tracers and advanced MS- and NMR-technologies. Our proteomics studies greatly benefit from the newly installed Global Q-ToF instrument allowing for *de novo* peptide sequencing. In

collaboration with the Polymer Department we aim to develop nanosensors for *in vivo* detection of specific metabolites and to study fungal growth in biomimetic nano- and microenvironments. The results will be used to develop nutrient efficient crop plants and stable cropping systems in order to preserve mineral nutrient resources and reduce production costs.

Research fields

- Functional diversity in transport, exchange and metabolism of nutrients in arbuscular mycorrhizas
- Pathways for phosphate uptake in responsive and non-responsive mycorrhizal plants
- Functional diversity of mycorrhizal fungi in relation to environmental perturbations (fungicide use and climate change)
- Selection of P efficient root hair mutants in mutagenised barley
- Development of polymer-based nanosensors for *in vivo* localisation and quantification of metabolites in cells and in sub-cellular compartments
- Biomimetic nano- and microenvironments for *in vitro* cultivation and functional studies of roots and mycorrhizal fungi
- The role of mycorrhizal fungi in phytostabilisation of radio-contaminated environments
- Proteomics as a tool to study the appressoria-specific proteins in *Phytophthora infestans*, a pathogen of potato
- Development of instrumental methods in plant biochemistry

Selected reports

- [Metabolic phosphate pools in arbuscular mycorrhizal fungi studied by *in vivo* ³¹P NMR spectroscopy](#)
- [High performance liquid chromatography on the nano-scale](#)

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Plant Products Programme

Objectives

The programme seeks to use modern technology to explore the inherited plasticity of plant organs to produce high value compounds and continuing improvement of quality and value of crop plants. Technologies are developed to convert a larger amount of natural products to compounds, which are more useful to industry (waste-to-value). Metabolic engineering will be used to control biosynthesis of secondary compounds and increase functionality of plant polymers and storage compounds.

Research fields

- [Plant mitochondria](#) with emphasis on plant-specific processes. Studies of the mitochondrial proteome in rice are focused on the identification of phospho-proteins in signal transduction and stress-related proteins.
- Plants response to biotic and abiotic stress, production of reactive oxygen species - ROS - and oxidized proteins in mitochondria and other compartments. The cause of stress includes salinity, pathogens and ozone.
- Plant fibres for new biodegradable materials and identification of high value products.
- Mutational breeding for improving the use of crop plants as biomass resource.
- Examination of starch biosynthesis with the aim of producing novel industrial products
- Phosphate uptake and grain phytate metabolism studied by induced mutation and in transgenic cereals. Inositol-phosphates are identified by metal-dye-detection-HPLC and enzymes important phytate turnover includes phytase and phosphatases.
- Biofortification and micronutrients

- Cereal seed development, nutrient loading and unloading and serine proteinase inhibitors involved in defense and control of regulatory pathways. Studies of seed specific promoters and establishing developmentally specific dbEST databases.
- Determination of metabolite concentrations as a tool to increase production through plant breeding.
- Crop plants: barley, rice, potato, flax
- Database on seed dbEST of finger millet

Selected reports

- [The fate of Zearalenone and Ochratoxin A in Soil](#)
- [Barley agmatine coumaroyltransferase produce precursors of antifungal factors](#)

Contact

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Resistance Biology Programme

Objectives

The Programme is conducting research on different aspects of plant-pathogens interaction. The background for this is that diseases have serious impact on plant productivity and therefore, growers are forced to use agrochemicals to control the damaging pathogens. However, this is expensive and possibly harmful to the environment and to human health. The objective of the Programme is to provide alternative measures for pathogen control. These primarily include exploitation of the plants natural resistance mechanisms, based either on introduction of new resistance genes or on genetic engineering. Another aim is to identify genes responsible for tolerance towards abiotic stresses. In order to meet these objectives on longer terms, we do research on the fundamental processes controlling plant-pathogen interaction, and e.g. drought tolerance. In addition, we study disease development in response to different cropping systems, and thereby we can give recommendations for agricultural practices in order to alleviate disease problems.

Research fields

- Cellular and molecular mechanisms of resistance to *Blumeria graminis* f.sp. *hordei* in barley and *Arabidopsis thaliana*
- Molecular cloning and characterization of barley genes involved in disease response to powdery mildew attack
- Molecular cloning and characterization of barley powdery mildew (*B. graminis* f.sp. *hordei*) stage-specific and avirulence genes
- Mapping of barley and wheat genes for disease resistance and abiotic stress resistance and development of marker-based selection systems
- Modelling dynamics of pathogen dispersal and interspecific competition, on wheat (yellow rust, *Puccinia striiformis* f.sp. *tritici*) and on barley (netblotch, *Pyrenophora teres*, and scald, *Rhynchosporium secalis*)
- Plant health and induced resistance in relation to variety mixtures, intercropping and nutrient uptake
- Proteomics of the peribacteroid membranes in legume nodules
- Crop improvement by means of biotechnology in developing countries
- Genetic resources for crops of the future

Selected reports

- [Syntaxin involved in penetration resistance](#)
- [Disease development in intercropping](#)

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Improving the nutritional value of pasture grass

Klaus K. Nielsen

DLF-Risø Biotechnology Programme

Keywords: Perennial ryegrass, *Lolium perenne*, nutritional value, fructans, *Brachypodium*, transformation.

The Biological Containment provided by the "non-flowering system" in development by the DLF-Risø Biotechnology Consortium allows growing grasses with a wide range of genetically improved qualitative traits. Recent research activities initiated by the Consortium include work on nutritional value and a monocot transformation platform for efficient testing of gene function.

Nutritional value

The nutritional value of grasses as a general trait becomes markedly reduced during the course of the growth season. This reduction is largely caused by an increase in the content of poorly digestible cell wall structural components together with a concomitant decrease in the content of soluble carbohydrates – "sugars". Varieties of ryegrass with a high stable level of carbohydrates in the form of fructans have been shown to retain a high degree of nutritional value throughout the growth season. Grass varieties with an increased level of soluble carbohydrates will lead to a more efficient uptake of proteins in ruminants, and thus, an enhanced milk and meat production.

Fructans are polyfructose molecules produced as major soluble storage carbohydrates in temperate grasses. Plant fructans vary in degree of polymerization and linkage type, depending on species and environmental conditions. Apart from their role in photosynthate partitioning, fructan accumulation has been associated with plant response to environmental stress such as cold and drought. Fructan biosynthesis in model plant species with well-defined sink organs is quite well characterized. In contrast, information on the genetic regulation of grass leaf fructan metabolism in leaves and leaf sheaths, serving as fructan storage compartments in grasses, is very limited. For this reason and because soluble carbohydrates are major determinants of forage quality, research on fructan metabolism is a research area of high priority to the Consortium.

The strategy to understand the genetic regulation of fructan metabolism in leaves of grasses involves the isolation and characterization of potential key genes in grass fructan biosynthesis and breakdown, including fructosyltransferases, invertases and exohydrolases. Genes are being isolated by means of differential screenings and sequence homology based screenings (in silico). The final test of candidate gene functions includes transgenic studies in ryegrass.

Monocot transformation platform

The Consortium is focusing on the establishment of a monocot transformation platform to serve as a novel model system allowing a fast and efficient testing of gene effects in grasses and cereals. The weedy grass species *Brachypodium distachyon* was chosen due to a number of unique features, making it a potential monocot counterpart to the widely used dicot model plant *Arabidopsis thaliana*.

Brachypodium belongs to the Pooideae subfamily like many of the agronomical important temperate cereals. It possesses unique monocot model plant characteristics in having the smallest known genome size in grasses of down to 123 Mbp/1C (similar to *Arabidopsis*), a life cycle of less than four months and a physical size of down to 20 cm at maturity.

Brachypodium is a self-fertile inbreeding annual with a chromosome base number of five. Di-, tetra- and hexaploid cytological genotypes have been collected mainly from South/East Europe.



Phenotypic appearance of *Brachypodium distacyon* belonging to the Pooideae subfamily



Brachypodium distacyon transformed with the *uidA* gene and showing high constitutive GUS activity

To establish *Brachypodium* as an efficient test bed for functional genomics in cereals and grasses, an efficient transformation system needs to be established. Published protocols have demonstrated the readiness of *Brachypodium* in respect to ballistic transformation, however, with insufficient efficiency and reproducibility. Since year 2000, the Biotechnology Consortium has performed activities on the development of an improved *Brachypodium* transformation system based on both ballistic and *Agrobacterium* mediated transformation, different selection markers, and employment of genotypes with a difference in ploidy levels, vernalisation requirements and phenotype characteristics. Presently the transformation systems are developed to an extent allowing routinely employment in PRD projects focused on testing of gene function. Future aims are to produce mutant collections, relevant cDNA and genomic libraries and to develop a basal genetic map.

Expression of a 434:VP16 chimeric activator leads to high-level activation of gene expression in Arabidopsis

Morten Storgaard

DLF-Risø Biotechnology Programme

(Storgaard et al. 2002: Trans. Research 11: 151-159).

Key words: 434:VP16 activator, activation, 434-repressor, repression, Arabidopsis, monocot promoter

Introduction

In order to design plants for future agricultural applications plant breeders require direct control of gene expression in crop plants. In addition to control of agronomic traits, manipulation of biosynthetic pathways has the potential to convert model plants into biological factories in which secondary metabolites are manipulated or products for non-food and pharmaceutical applications are produced. Controlled gene expression can be achieved by several mechanisms for example, by regulation of gene expression in a chemical dependent manner. Unfortunately, most of the existing systems are unsuitable for agricultural use because of known or putative harmful effects of the chemicals used to induce gene expression.

Controlled gene expression can also be achieved by a genetic switch based on a hybrid system, where one parental line (the activator line) expresses an activator protein in a constitutive manner and the other parent (the reporter line) harbors the gene of interest under the control of a promoter which only can be activated by the specific activator expressed in the activator line. This mechanism allows phenotypically normal transgenic lines to be generated and propagated without interference from the gene of interest.

Crossing the activator and reporter lines results in binding of the activator to specific operator elements in the promoter thereby stimulating expression of the gene of interest. In order to avoid expression of the transgene already in the reporter line alone, the choice of the activator together with the corresponding operator elements is crucial. Therefore, the use of transcription factors with sequence-specific DNA-binding activities not found in plants is a prerequisite. Several chimeric transcription factors successfully used in stable transformed plants are already described, including the GAL4:VP16 (Schwechheimer, et al. 1998), the GAL:C1 (Guyer et al., 1998) and the LacI:GAL4 (Moore et al., 1998) fusion proteins.

Expression of a 434:VP16 chimeric activator

The performance of an expression system based on a fusion of the bacteriophage 434-repressor to the VP16 activation domain of *Herpes simplex virus* type 1 (434:VP16) was tested after stable integration into *Arabidopsis*. A special feature of this system was the use of the monocot maize ubiquitin 1 and rice actin promoters to drive the expression of the 434:VP16 activator and 434-repressor, respectively. Our results demonstrated that the maize ubiquitin 1 and the rice actin promoters, each of which contain introns, are active in *Arabidopsis* and can be used to express genes in this dicot species. Activation of gene expression after co-integration of the activator and reporter cassettes into the same genomic locus (figure 1-A) resulted in a higher activation level (84-fold activation) compared to crossing individual lines (figure 1-B) expressing only the activator or the operator reporter cassette alone (8-fold activation). Increasing the number of operator elements in the reporter cassette from one to four increased the activation level in cross-activated lines to an average of 90-fold with one combination of parental lines gives a 900-fold activation. Simultaneous expression of the 434-repressor protein driven by the rice actin promoter resulted in a significant decrease in the 434:VP16 mediated reporter gene expression. Nevertheless, an overall induction via 434:VP16 was possible even in the presence of the 434-repressor protein (figure 1-C). This feature is important for genes required to be absolutely repressed except under activating conditions. To our knowledge this investigation is the first report on the use of the 434:VP16 chimeric activator in an expression system in stably transformed plant lines.

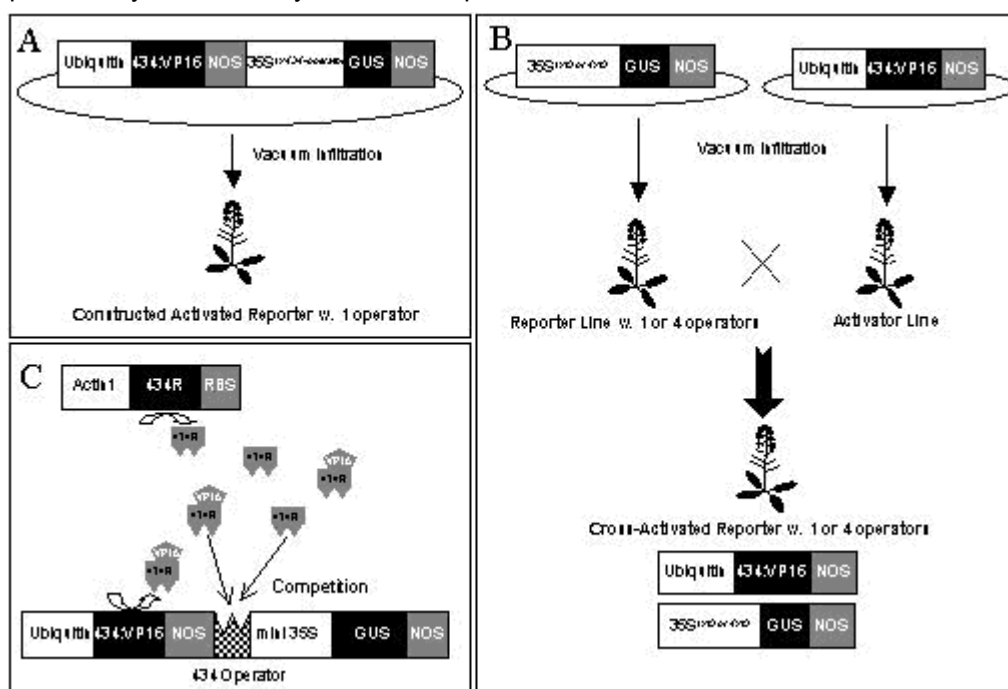


Figure 1.

(A) Schematic illustration of the generation of constructed activated reporter lines with one operator. The activator and reporter cassettes were combined on one plasmid and transformed into *Arabidopsis*. Activator cassette and reporter cassette integrate at the same locus into the *Arabidopsis* genome.

(B) Schematic illustration of the generation of cross-activated plant lines. The activator cassette and reporter cassettes (with one or four operator elements linked to the minimal

M35S promoter GUS fusion) on separated plasmids were each transformed into *Arabidopsis* thereby generating reporter lines with one or four operator elements and activator lines. Crossing of an activator line to a reporter line resulted in cross-activated reporter lines with activator and reporter cassettes integrated at different loci in the *Arabidopsis* genome.

(C) Schematic illustration of the plant lines expressing both the chimeric 434/VP16 activator and the 434 repressor. The plant lines were generated via crossing of a constructed activated reporter line with one operator to a line expressing the 434 repressor protein from a rice actin promoter. The simultaneous expression of the 434/VP16 chimeric activator protein and the 434 repressor protein resulted in a competition of chimeric activator and repressor for the binding to the 434 operator element.

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Responses to elevated CO₂ and drought-stress in old and new varieties of oats, *Avena sativa*

Marina Mosbæk Johannessen, Teis Nørgaard Mikkelsen and Rikke Bagger Jørgensen

Plant-Environment Interactions Programme

Our environment is changing because of climate changes and pollution. Thus, besides adjustment to the management system, the development of future crops must be oriented towards the future environment, since there will be new demands for the "crops of tomorrow". Breeding is time consuming and a long-term process, and it is therefore of high emergence to gain knowledge about traits in old and new varieties, since their genetic resources could be an advantage or maybe a prerequisite in future agriculture.

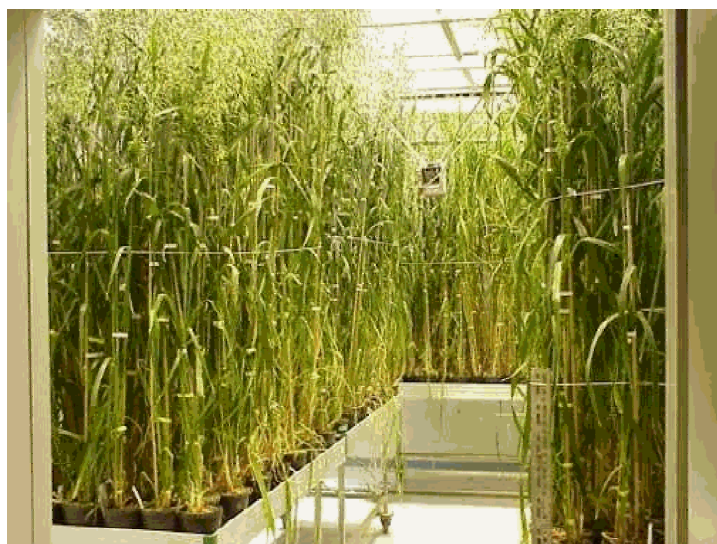
During the last 80 years the CO₂ concentration in the atmosphere has increased from 300 parts per million (ppm) to 370 ppm. In the next 80 years, it is predicted that the concentration will double its current level to approximately 700 ppm. The temperature is expected to raise 3° C within the next century, thereby increasing the evaporation leading to longer or more frequent spells of drought.

These rapid changes affect the growth of plants in many aspects, but it is generally assumed that an increase in atmospheric CO₂ will e.g. accelerate growth and increase seed yield, whereas drought will have the opposite effect. Previous studies of the combined effect of elevated CO₂ and drought has shown that the severe effect of drought is reduced when there are plenty of CO₂ available, because plants can achieve enough CO₂ with reduced stomatal openings, and then retain their water for longer periods. We investigated how seven different Scandinavian varieties of oats (three old (before 1920), two of intermediate-age (1920-1940), and two new varieties (after 1940)) responded to two different CO₂ regimes (370 and 700 ppm). Seed material was obtained from the Nordic gene bank. Two varieties were also compared in a combined CO₂-drought treatment lasting for seven days during the growth period.

The study was conducted in two gas tight chambers in RERAF. Besides the CO₂ and drought exposure the plants were treated identically. A daily and seasonal variation in light, temperature and humidity were programmed simulating natural growth conditions. Wind is a factor of great importance for the water-budget, thus to achieve a closer to natural situation wind-turbines were placed in the chambers.

Some of the parameters investigated during the treatments were: heading of the first spike, the relative chlorophyll content and gas exchange. After harvest, the biomass components seed-yield, total seed number, seed weight and vegetative dry biomass were determined.

The yield increased on average about 7% under elevated CO_2 conditions, which is consistent with the expectation, but we found very large variation between varieties. All old varieties were higher yielding at elevated CO_2 , whereas the intermediate-age and new varieties had a representative of a lower and a higher yielding variety. An ANOVA confirmed that the response in yield was completely variety specific, not age-group specific. The mean seed number increased about 11%, resulting from a general increase in the old varieties and diverging responses within the two other age-groups. The mean seed weight was reduced about 5.5%, with small changes within the old varieties and diverging results within each of the two other age-groups. The mean dry biomass was increased about 20%. The old varieties all had a marked increase in dry biomass, while the response within the intermediate-age varieties, ranged from a large increase to a small decrease. The new varieties had a small or close to zero increase in dry biomass. These very different response patterns in biomass-components among varieties may make it possible to select a certain composition of these features for future breeding.



The heading of the first spike as well as other developmental markers is not informative in respect to comparing growth rates between varieties, only within varieties, because varieties may have different strategies for development. An ANOVA revealed no significant difference in heading between the CO_2 levels. Thus the elevated CO_2 concentration did not accelerate the growth of oats.

The stomatal conductance (g_s) and the maximum photosynthesis capacity (A_{max}) were measured during the spell of drought. In general the stomatal conductance is expected to decrease about 30% with a doubling of the CO_2 concentration. During drought this change is expected to be more pronounced. The maximum photosynthesis is expected to increase considerably when the CO_2 level is elevated and to be more moderate during drought. In our experiment there was an average decrease in stomatal conductance of about 19% without the drought-treatment and a 28% reduction on average during the spell of drought. The increase in maximum photosynthesis capacity was 82% without drought stress and 73% with. There was a significant variety and day effect on both dependent variables. The combined treatment also had a significant effect, and was in accordance with the data to a larger extent for the A_{max} , than when they were included as separate terms. For the g_s the separate terms explained the data as well as the combined term.

Our previous results on CO_2 exploitation in oilseed rape, also showed very different responses between varieties. In the two different crop species that we analysed there is a general tendency for old varieties to respond more heavily, whereas younger varieties are more unpredictable. The two crop species are different in that oilseed rape accelerates its growth in contrast to oats. Thus since crop plants respond in different ways to "global

change conditions", it is of importance that their response is evaluated on a "crop by crop basis" and maybe also a "variety by variety basis".

Effects of UV-B radiation on the high arctic vegetation in Northeast Greenland

Teis N. Mikkelsen¹, Helge Ro-Poulsen² & Linda Bredahl²

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2) **University of Copenhagen**

Risø and University of Copenhagen conducts a study of UV-B effects on the high arctic vegetation at Zackenberg, Northeast Greenland.

Due to the degradation of the ozone layer, the ambient UV-B radiation will increase in the next 15-20 years (www.DMI.dk). UV-B is a stress factor on plant growth and competition abilities. Plants can adjust to environments with higher UV-B levels – partly by genetic selection and partly by acclimation. Plant protection can be the development of thickened surfaces and encasement of more pigments with UV-B absorption. If the UV-B protection is insufficient the genome and the photosynthesis apparatus will be damaged, and repair processes will be activated. Protection and repair processes need allocation of energy and substances that will reduce plant growth and reproduction potential. It can be very critical for the arctic vegetation, because it is very vulnerable to stress factors that reduce energy utilization. Uptake of energy is a limitation factor because of the very short growth season of two months.

The experiment

In July 2001 in Northeast Zackenberg, Greenland. (74°3'N, 20°3' W) a 3 year UV-B exclusion experiments was started on arctic heathlands. We investigate effects of the current UV-B radiation in relation to reductions in UV-B. Metal frames (47x60 cm) with transparent films of different UV-B absorptions characteristics were placed over the vegetation on the south slopes of the surrounding hills.

The following treatments were given:

- Filter, reduction of UV-B to 17% of ambient
- Filter, reduction of UV-B to 40% of ambient
- Filter, reduction of UV-B to 92% of ambient
- No filter (control) 100% UV-B

The experiment was conducted at two sites with four replicates of each treatment (figure 1). The potential and actual CO₂ uptake was studied by the use of induced chlorophyll fluorescence and gas exchange. Two plants species (*Vaccinium uliginosum* og *Salix arctica*) were investigated during the full growth season from the beginning of July to late August.



Figure 1. A view over a part of Site 1. Frames with transparent filters cover a part of the vegetation and reduces the incoming UV-B radiation. The white box contains a data logger that measures air and soil temperature under the frames, soil water content, air humidity, and incoming light.

Results

In 2001, the leaves that received the highest levels of UV-B were stressed by UV-B, compared to the leaves that received reduced levels of UV-B. Shown in figure 2 are reductions in the potential CO₂ uptake throughout the season. Fv/Fm illustrates the level of stress in the leaves, high Fv/Fm = low levels of stress, and low Fv/Fm = high levels of stress. On days with high sun irradiation effects are more pronounced. No significant differences were seen for the gas exchange measurements. This could be associated to the fact that the CO₂ fixing processes are not capable of taking advantage of the stress relief, or it could be related to the low sample size. Another variable that increased the variability is that each leaf received different UV-B doses related to the leaf and sun angle. In all treatments, the CO₂ assimilation shows clear seasonal variations. The leaves increase the maximal potential CO₂ uptake until late July, after which the uptake decreases due to initiation of senescence. The data processing of the 2002 data is not complete yet, but preliminary results indicate that differences between treatments are less pronounced. Whether this is caused by acclimation or the lower influx of light in 2002 still has to be clarified.

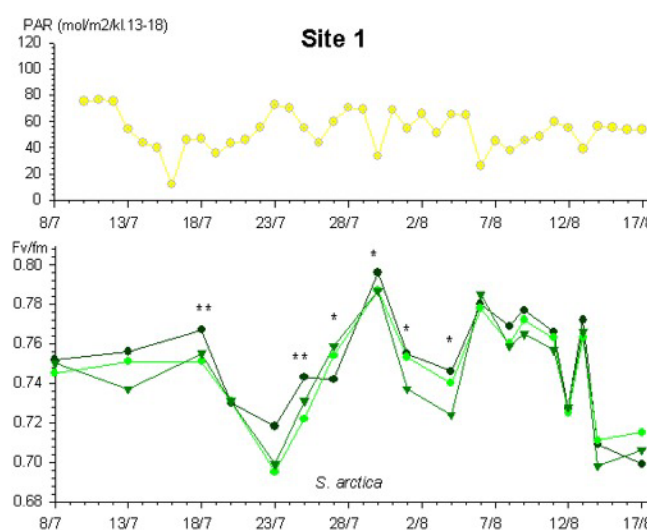


Figure 2. Potential CO₂ uptake (Fv/Fm) per treatment per day for *Salix Arctica* and daily accumulated Photosynthetic Active Radiation on Site 1. On single days, significant differences are seen between the treatments (two-factor ANOVA). Days with significant differences are marked with * when $p < 0.05$ and ** when $p < 0.01$. Dark green = 17% of ambient UV-B; medium green = 40% of ambient UV-B; light green = 92% of ambient UV-B.

Metabolic phosphate pools in arbuscular mycorrhizal fungi studied by *in vivo* ³¹P NMR spectroscopy

Nanna Viereck

Plant Nutrition Programme

AM fungi colonize the roots of most land plants and the symbiosis between AM fungi and plants is characterized by bi-directional nutrient transport; the AM fungus receiving fixed carbon in return for improved inorganic nutrient (mainly P) uptake by the host root. The extraradical mycelium of an AM fungus forms an extensive hyphal network surrounding the roots and allows the plant to access P_i further out in the soil. Once the association is established, the fungus takes up P_i from the soil through the extraradical mycelium in an active process like plants. However, AM fungi also accumulate P_i as polyP, which is believed to be translocated to the intraradical mycelium in vacuoles in a tubular streaming system. Accordingly, polyP is considered to have an important role in the translocation process. However, the amount, size and possible other roles of polyP present in the fungus is a matter of debate. We have investigated the dynamic incorporation of P_i into

various P pools within extraradical mycelium and mycorrhizal roots by the non-invasive *in vivo* ^{31}P NMR spectroscopy and by this the dynamics of polyP synthesis.

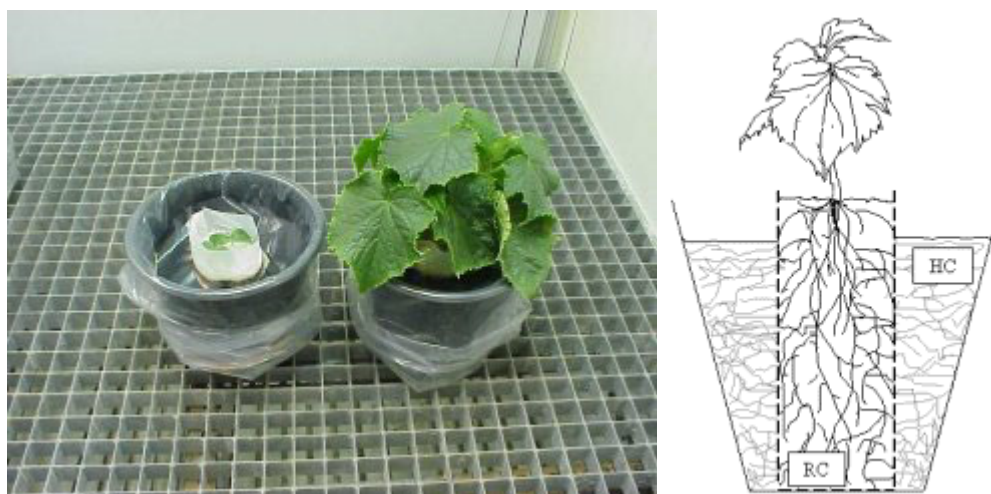


Fig. 1. Compartmented growth system, composed of a soil filled mesh-bag (root compartment, RC) surrounded by sand (hyphal compartment, HC).

The biological material chosen was cucumber grown in symbiosis with the AM fungus *Glomus intraradices* in a compartmented growth system (Fig. 1). The cucumber plants were grown in a soil filled mesh-bag, which prevents root penetration but allow free passage of AM fungal hyphae. The extraradical mycelium grew into the surrounding sand and could be collected from this, while root material could be collected from the mesh-bag. An air-circulation system was constructed for oxygenating the excised hyphae or roots while in the NMR tube.

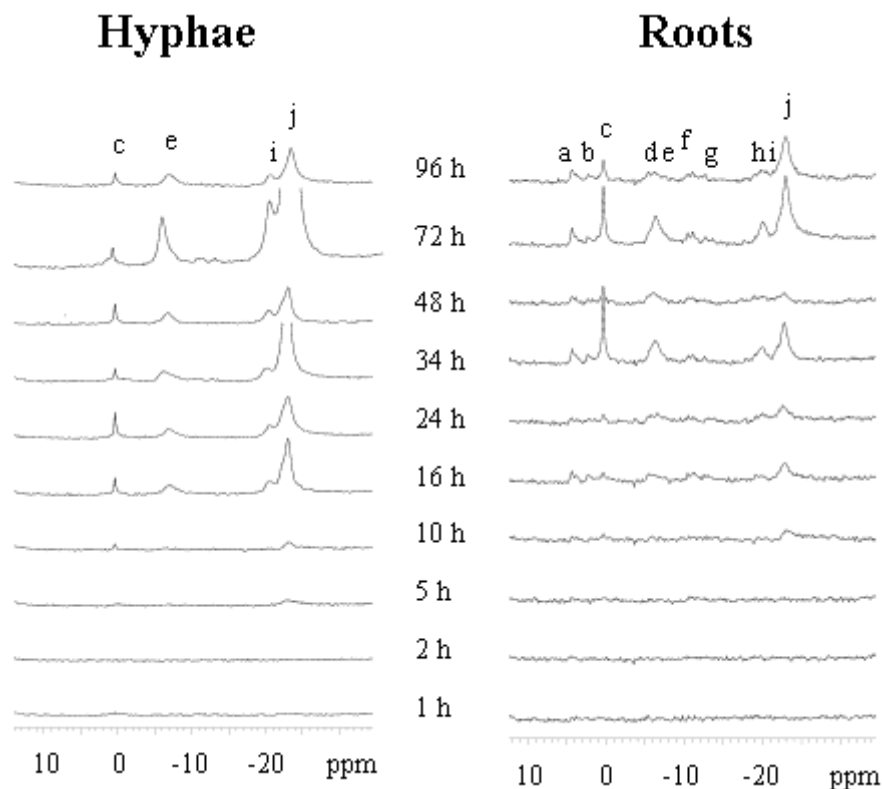


Fig. 2. *In vivo* ^{31}P NMR spectra of excised *Glomus intraradices* hyphae and mycorrhizal cucumber roots from pots harvested individually 10 times after a supply of $100 \text{ mg P pot}^{-1}$ showing a time-course of the formation of P pools. Peak assignments were as follows: (a),

several phosphomonoesters; (b), cytoplasmic P_i ; (c), vacuolar P_i ; (d), γ -NTP; (e), terminal polyP residues; (f), α -NTP; (g), uridine diphosphoglucose; (h), β -NTP; (i), penultimate polyP residues; (j), central polyP residues.

In this study, polyP of a short chain length was seen in actively metabolizing extraradical AM fungal hyphae for the first time by the use of *in vivo* ^{31}P NMR spectroscopy. Furthermore, a time-course ^{31}P NMR investigation of the formation of P pools in differently P-treated AM hyphae and mycorrhizal roots was performed (Fig. 2). It was demonstrated that P_i taken up by the extraradical mycelium accumulated firstly into polyP and subsequently into vacuolar P_i within the extraradical mycelium. A time lag was observed before any P metabolites appeared in mycorrhizal roots. The amount of polyP in extraradical mycelium was considerably higher than vacuolar P_i and synthesis of polyP was therefore suggested to be important for effective P_i uptake in AM fungi. The chemical shift of the signal from the terminal P_i residues in the polyP chain is pH dependent and a calibration curve can therefore be used to identify the intracellular compartment for the polyP. The pH of the compartment was found to be approximately 6 (Fig. 3) which predicts the polyP to be located in the acidic vacuoles. The average chain length of the polyP can be determined from the ^{31}P NMR spectrum and was measured to be short (< 20 P_i residues). A high level of vacuolar short chain polyP is supporting an important role for polyP in effective translocation of P_i from soil to host root by AM fungi. Cytoplasmic P_i in the extraradical mycelium could not be detected by *in vivo* ^{31}P NMR, possibly because of a small cytoplasmic volume or a low concentration of cytoplasmic P_i .

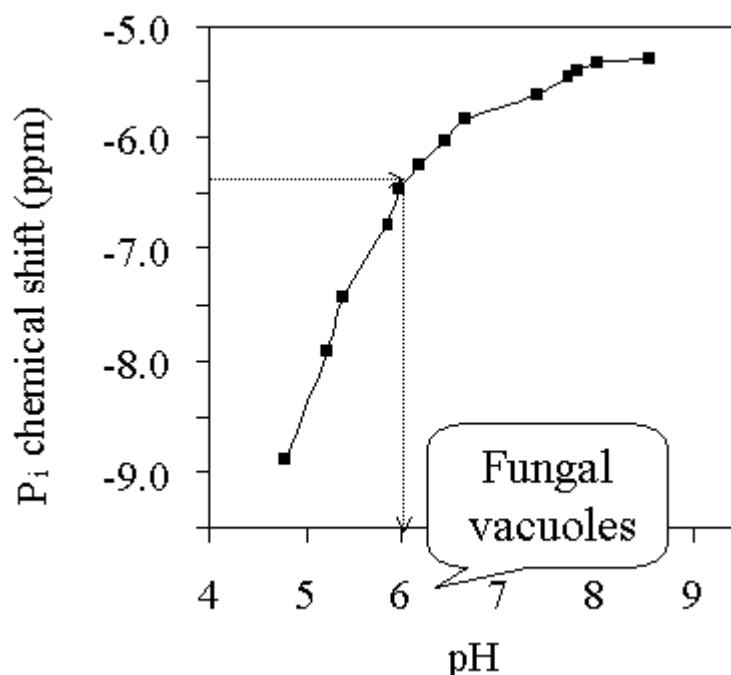


Fig. 3. The pH of the intracellular compartment for the fungal polyP was determined from the chemical shift of the terminal P_i residues in the polyP chain.

The average polyP chain length was further characterized by the use of extraction procedures and colorimetric measurements. Combining the results obtained from these methods and NMR revealed small amounts of long-chain and granular polyP in the extraradical mycelium when supplied with high P amounts.

In conclusion, we have shown that *in vivo* ^{31}P NMR spectroscopy is a valuable method for identifying and semi-quantifying various P metabolites including polyP in the AM symbiosis but a combination of methods seem favorable for studies of polyP chain lengths.

High performance liquid chromatography on the nano-scale

Helge Egsgaard

Plant Nutrition Programme

The Plant Research Department routinely uses electro-spray ionisation mass spectrometry in proteome research. Electro-spray ionisation is a highly efficient ionisation method for peptides, where the ionisation process takes place directly in solution by control of pH. Thus, interfacing liquid chromatography (LC) and mass spectrometry becomes straightforward. An important feature of the electro-spray ionisation method is the increase in sensitivity when lowering the liquid flow rate. Thus, the application of nano-technology has in this context a significant advantage and dedicated nano-LC systems have already been developed.

The Plant Research Department has in 2002 purchased two nano-LC systems from LC-Packings to be used for proteomics. The systems have a gradient pump system programmable in steps of 10 nL/min. Samples are loaded by a micro-autosampler capable of handling samples from 50 nL. Samples are loaded to a pre-column and back-flushed to the final analytical column. The latter is a packed capillary (75 μ m id x 15 cm). The column switching systems controlling the flow in the pre-column and the analytical column enable advanced strategies, e.g. 2-dimensional chromatography based on the combination of ion exchange columns and reverse phase columns. In addition, UV-detection with a 3 nL flow-cell and peak-parking is on hand.



Fig 1. LC Packings nano-LC system.

The nano-LC systems are interfaced directly to a ThermoFinnigan LCQ and a Micromass Qtof Ultima Global mass spectrometer, respectively. In Figure 1 is shown the complete nano-LC as interfaced to the LCQ mass spectrometer. The performance of the system is briefly illustrated in Figure 2 with the analysis of the peptides released from a single spot cut from a standard 2D-gel analysis of proteins following in-gel enzymatic digestion. The upper trace corresponds to the MS signal generated in the data dependent acquisition. The detection limit in this trace is on the order 10 femtomol. The peptides elute in the time window 20-35 min. The lower trace is the m/z 1178.9 ion chromatogram and illustrates the chromatography of the H LAPFLPSHPVITGGFPLPEK peptide

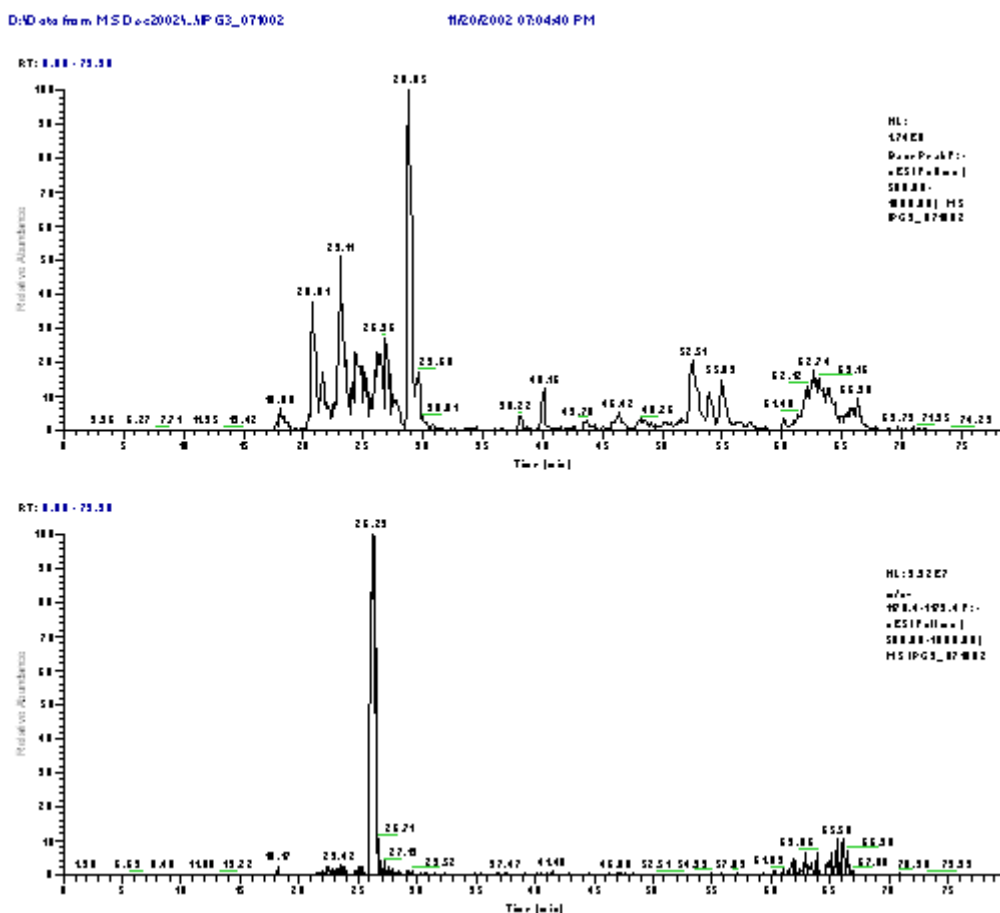


Fig 2. Analysis of in-gel tryptic digest (MS signal generated in the data depending acquisition (top) and m/z 1178.9 ion chromatogram (bottom)).

Q-ToF Ultima Global

Nutrition Programme

A Q-ToF Ultima Global mass spectrometer was acquired and became operational ultimo 2002. The instrument allows fast switching between electro-spray and MALDI (matrix assisted laser desorption and ionization) modes and, hence, delivers ideal flexibility to the biochemical research environment. The instrument features Woptics, boosting the dynamic resolution by a factor two. Software enables data- directed analysis for the discovery and analysis of precursor ions with unprecedented speed and selectivity. This is of key importance in our on-line 2-dimensional chromatographic analysis of complex peptide mixtures, cf. Figure 1.



Figure 1. nano-LC coupled to the Q-ToF mass spectrometer.

The fate of Zearalenone and Ochratoxin A in Soil

Gerda Krog Mortensen

Plant Products Programme

Mycotoxins are secondary metabolites, formed by the action of fungi on agricultural crops in the field or during storage. *Penicillium* species are generally regarded as storage fungi but studies have shown that *P. verrucosum* can survive in Danish field soils and become an integral part of the soil ecosystem. In Danish grown small cereals, ochratoxin A (OTA) and zearalenone (ZON) and trichothecenes are considered to be the most important mycotoxins. Under Danish conditions, OTA is produced by *P. verrucosum* and ZON and trichothecenes by different species of *Fusarium*, mainly *F. graminearum*. These metabolites are highly toxic to animals and humans and high contents have been measured in agricultural crops from many different countries. Up to 8 mg kg⁻¹ ZON has been measured in wheat from Germany and OTA has been measured in different crops up to about 20 µg kg⁻¹.

But almost no research has been done in relation to the fate of natural toxins in the soil-plant system and investigation of possible risk for groundwater contamination. In a project together with The Royal Veterinary and Agricultural School (KVL) the focus was on mycotoxins in agricultural soil and the fate of these toxins in the soil-water-plant system. Two different mycotoxins were selected in the study: ZON and OTA. The structures of these toxins are shown in Figure 1.

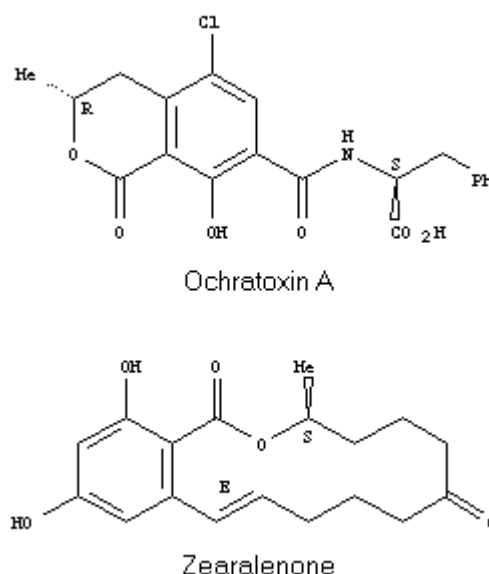


Figure 1: The structure of OTA and ZON

Although the toxins consist of aromatic structures, the hydroxy and carboxylic groups increase the solubility in water of these compounds compared to other organic contaminants. Solubility in water about 20 mg L^{-1} has been reported for ZON. We have developed a method for analysis of these toxins in soil with detection limits at 0.1 and $1.0 \mu\text{g kg}^{-1}$ dry weight (dw) for OTA and ZON, respectively. These detection limits are comparable or below the detection limits reported for plant materials. The developed method has been used for different soil analysis in connection to soil adsorption experiments and degradation studies in growth chambers. The soil types used in the growth chamber experiments were a sandy soil, a sandy clay soil and a soil with high content of organic matter from Jyndevad, Askov and Lammefjorden, respectively. Adsorption of ZON to soil was determined in a master project by Stine Hallberg Hansen. These batch experiments were performed in accordance with OECD Guideline no. 106 and are useful for generating essential information on the mobility of chemicals and their distribution in the soil. The adsorption of ZON could be described by the Freundlich equation and the calculated parameters together with selected soil parameters are shown in Table 1. The distribution coefficients (K_d) show the importance of the soil type where the highest fraction of ZON in soil compared to water is measured for the organic soil. And these factors also influence the adsorption constant K_F , which also is highest for the organic soil.

Table 1

Calculated constants for zearalenone adsorption in soil

Soil	Organic C %	Clay %	K_d L/kg	K_F L/kg
Jyndevad	2.05	3.9	54	19
Askov	4.54	10.6	101	35
Lammefjord	6.84	4.4	154	54

Degradation of ZON and OTA in soil was investigated in experiments with the same soil types. Mycotoxins were spiked into the soil and soil samples were taken during a period of 225 days. There was also experiments with soil planted with barley.

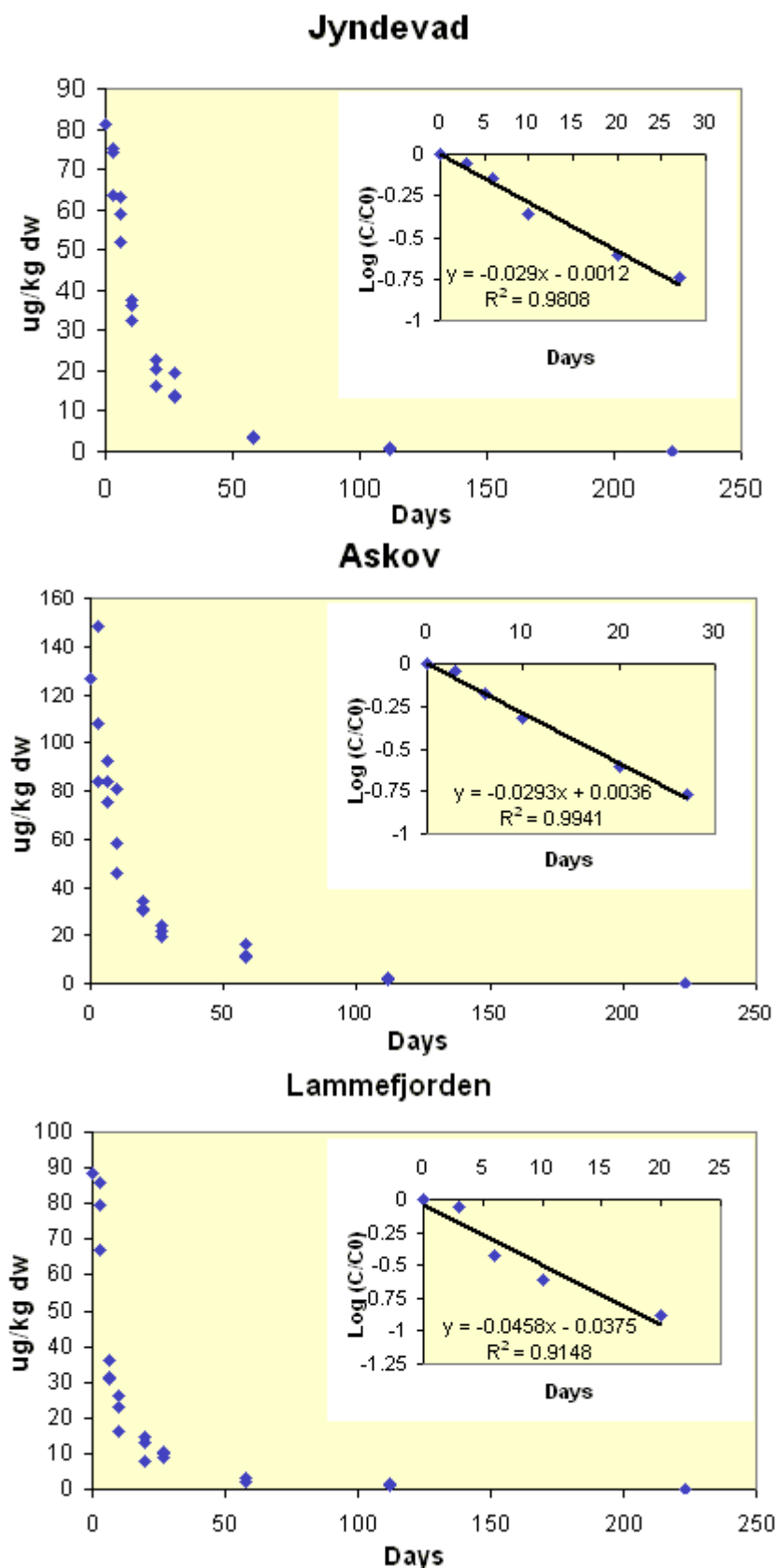


Figure 2: Degradation of ZON

In Figure 2 the degradation of ZON is shown in soil without plant growth. There was a fast degradation where the first phase can be described as a first order degradation. The degradation in the organic soil was faster compared to the other soil types. And after 6

days just 36% was still in the soil compared to 65% and 70%. After 225 days there was no detectable contents in all soil types. OTA was degraded faster than ZON. After 6 days 3%, 9% and 25% was in the soil for Lammefjorden, Jyndevad and Askov, respectively. For both toxins the degradation was faster in planted soil probably due to higher microbial activity.

Conclusion

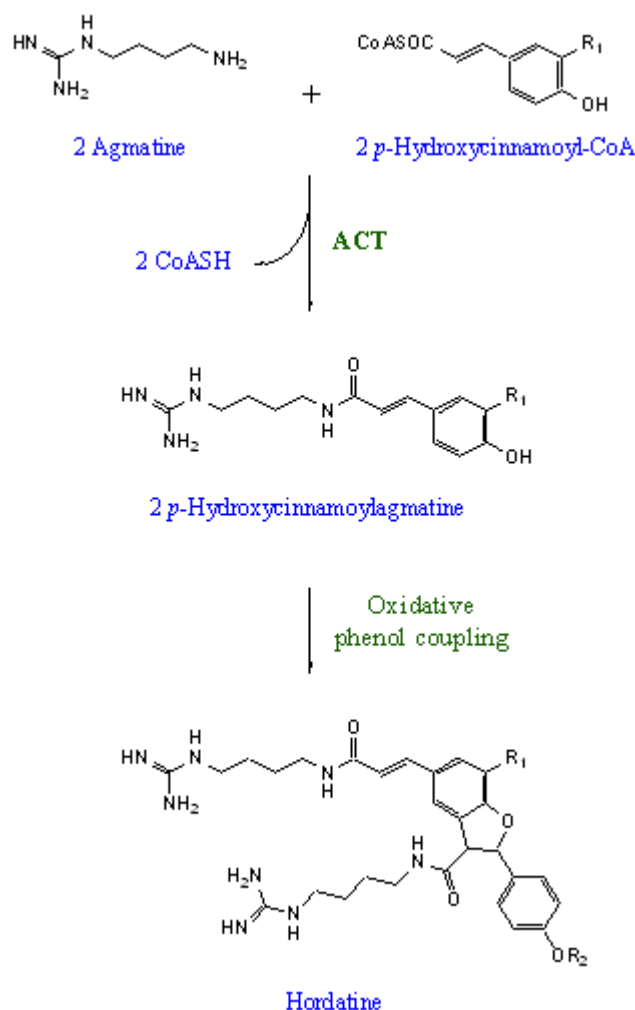
Generally, the mycotoxins are stable contaminants when they are incorporated in grain, other plant parts or food products. But in the soil-plant systems they are degraded and for these two toxins there seems not to be problems in relation to mobility and groundwater contamination.

Barley agmatine coumaroyltransferase produce precursors of antifungal factors

Kim Burhenne, Brian K. Kristensen and Søren K. Rasmussen

Plant Products Programme

Agmatine coumaroyltransferase (ACT, EC 2.3.1.64) catalyses the synthesis of hydroxycinnamoylagmatines from agmatine and hydroxycinnamoyl-CoA thioesters in barley (*Hordeum vulgare*). Hydroxycinnamoylagmatines are direct precursor of hordatines (Fig. 1), which are antifungal compounds found to be highly abundant in the young barley seedling. The hordatines seem to be confined to the genus *Hordeum* as preformed infectional inhibitors, and recent studies indicate that the synthesis of hydroxycinnamoylagmatine derivatives are induced in response to fungal infection of the leaves. Additionally, hydroxycinnamoylagmatine derivatives have been found in wheat, and histochemical staining of epidermal leaf tissue confirms that these compounds might accumulate in cereals in general as a response to fungal infection. The function(s) of hydroxycinnamoylagmatine derivatives in plants is not known, but may include cell wall fortification, restriction of pathogen ingress, and being cytotoxic to the invading pathogen. Related hydroxycinnamic acid amides are found throughout the Plant Kingdom and three plant N-hydroxycinnamoyltransferases have been purified and characterised: tyramine N-hydroxycinnamoyltransferase (THT, EC 2.3.1.110), putrescine N-hydroxycinnamoyltransferase (PHT, EC 2.3.1.138) and anthranilate N-hydroxycinnamoyl/benzoyltransferase (HCBT, EC 2.3.1.144). In general, the role of hydroxycinnamic acid amides remains speculative, but conjugation compounds of tyramine have been repeatedly reported to be present in cell wall fractions from several plant species and they are believed to produce a phenolic barrier against pathogens by reducing cell wall digestibility and forming the phenolic domain of suberin. However, anthranilic acid amides have been predominantly detected as soluble compounds and their synthesis can be induced in response to pathogen attack. The purified transferases are all cytosolic proteins and the carnation HCBT cDNA and THT cDNAs from tobacco, potato and red pepper have been cloned. The amino acid sequence identity is high between the characterised THTs, but the HCBT shares an absolute minimum of sequence similarity to the THTs. Thus, although these acyltransferases have a number of related enzymatic properties, the primary structure of the enzymes differs considerably. In order to enable further analysis of the hydroxycinnamoylagmatine derivatives, and their synthesis and potential significance in plant defence, a biochemical approach was taken to enable molecular characterisation of ACT.



acyltransferases indicated the existence of four evolutionary groups (A-D) but by including barley ACT we propose a fifth evolutionary group (E). Group A includes acyltransferases, which transfer hydroxycinnamoyl groups to acceptors from the shikimate pathway. Acyltransferases of group B and C are involved in taxol or anthocyanidin biosynthesis, respectively. Group D comprise acyltransferases that esterify a hydroxyl moiety of metabolic unrelated molecules. This group might define acyltransferases transferring hydroxycinnamoyl groups to acceptors derived from the polyamine pathway. Hydroxycinnamic acid amides of di- and polyamines are widely distributed in the Plant Kingdom. The amino acid sequence of pHVACT-5-28-6 shows 75% and 38% identity to the two rice proteins that ACT congregates with in Fig. 6. Proteins in group A show 27-29% identity and the transferases of the other groups (B-E) show less than 20% identity.

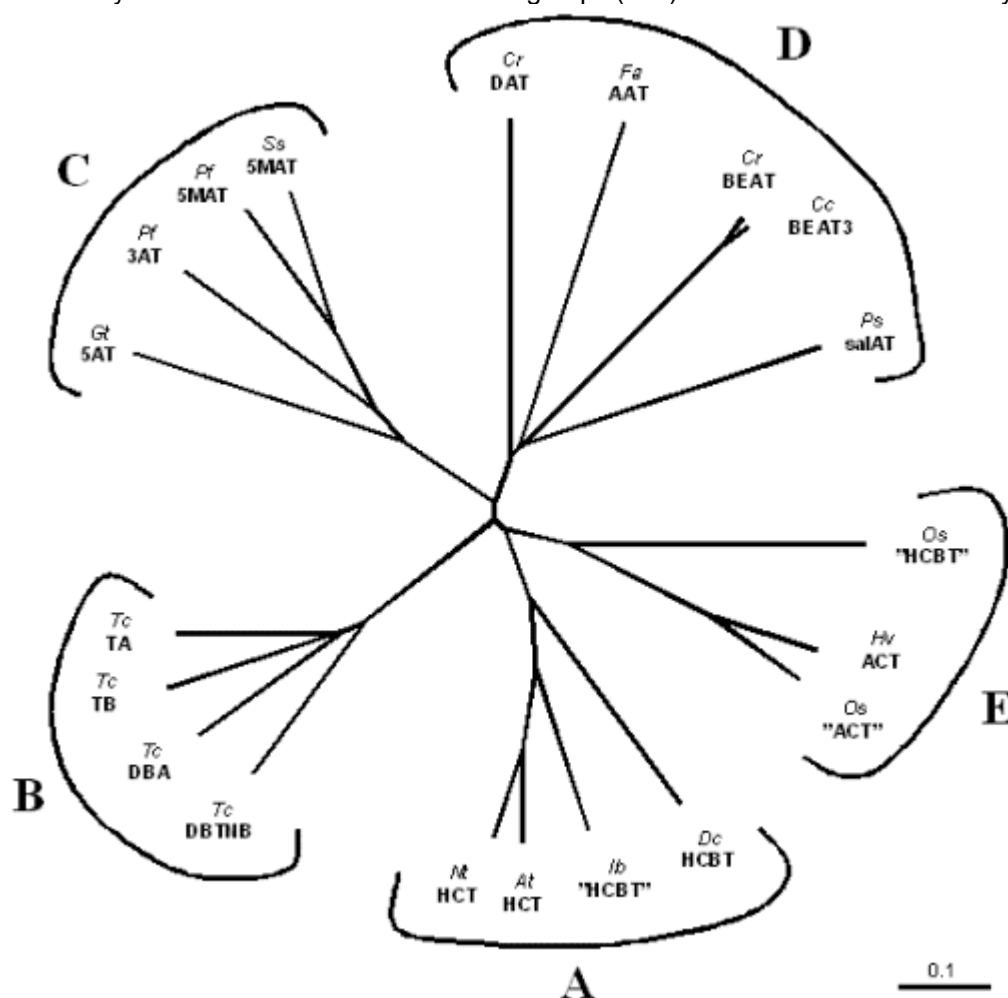


Figure 2

Phylogenetic analysis of acyltransferases indicating five evolutionary sequence clusters. The tree was constructed by the neighbor-joining method. The lengths of lines indicate the relative distances between nodes.

Reference:

K. Burhenne, B. K. Kristensen and S. K. Rasmussen: A new class of N-hydroxycinnamoyltransferases: purification, cloning and expression of a barley agmatine coumaroyltransferase (EC 2.3.1.64). J. Biol. Chem. 278 (16), 13919-13927.

Genetic evidence for the involvement of vesicle trafficking in non-host resistance of *Arabidopsis* to the barley powdery mildew fungus

Hans Thordal-Christensen, Jin-long Qiu and Helge Tippmann

Resistance Biology Programme

Plants defend themselves towards pathogens using a multitude of molecular mechanisms. This is true both for host and non-host plants. A non-host plant is defined as a plant species, which never is diseased by a pathogen known to cause disease on another (host) species. In many cases, non-host situations are considered to be due to infallible recognition of all genotypes of an invader, and subsequent activation of efficient defence mechanisms. Therefore, studying non-host resistance can result in knowledge about efficient defence mechanisms as well as on how the non-host pathogen recognition takes place. Both phenomena have potentials in practical disease control.

We study the non-host interaction between the model plant species *Arabidopsis* and the barley powdery mildew fungus. The model plant provides many molecular and genetics tools, useful for detailed analyses of defence processes towards this fungal pathogen, which is the focus of several projects in our programme. A mutant screen has been conducted in order to identify plants with hampered non-host resistance. Two mutant lines were identified which have reduced penetration resistance. The frequency by which the powdery mildew spores penetrate the epidermal cell wall increases from about 20% in wild-type to about 80% in the two mutants. The two lines turned out to be mutated in the same gene, which was named *PEN1*. *PEN1* was identified by map-based cloning to encode a protein of the syntaxin-family. Syntaxins play an important role in vesicle trafficking within the cell, where they are anchored in the membrane of acceptor compartments, which receive vesicles from the diverse donor compartments. The syntaxins interact with proteins on the vesicles and the membrane to form a four helix complex, which drive the vesicles and the target membrane to merge, and thereby allow the cargo of the vesicle to be delivered on the reverse side of the membrane (Fig. 1). *Arabidopsis* has 24 syntaxins, and they are thought to be involved in the movement of different types of vesicles of the cell. By generating a transgenic line with a gene construct for the *PEN1*-protein, fused by the N-terminal to the green fluorescent protein (GFP), we provide evidence that this particular syntaxin, as expected, is located at the plasma-membrane (Fig. 2).

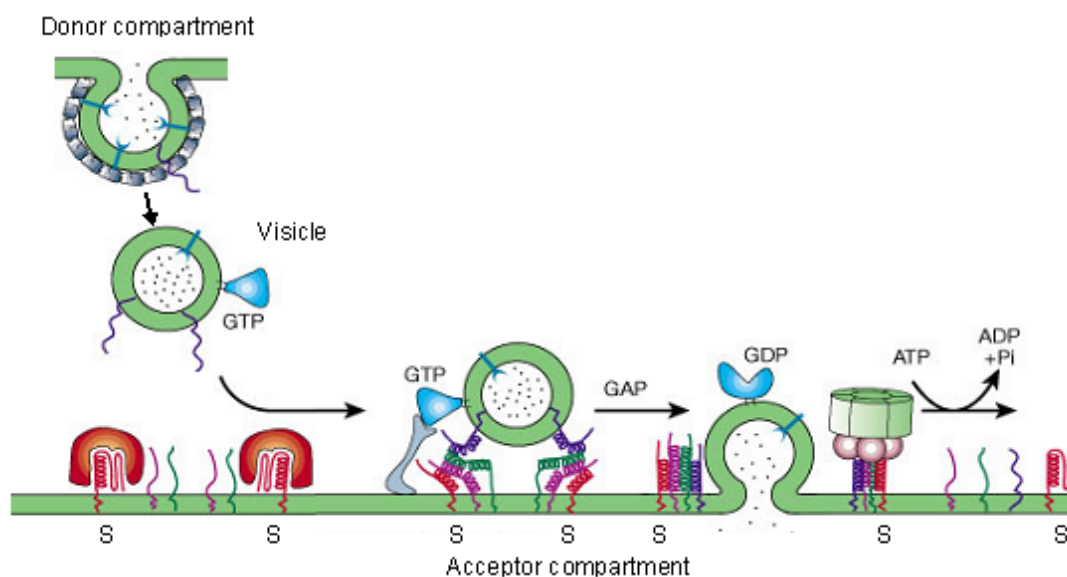


Figure 1. Syntaxin (S) is involved in cellular vesicle trafficking. From Bock et al., *Nature* (2001) 409, 839-841.

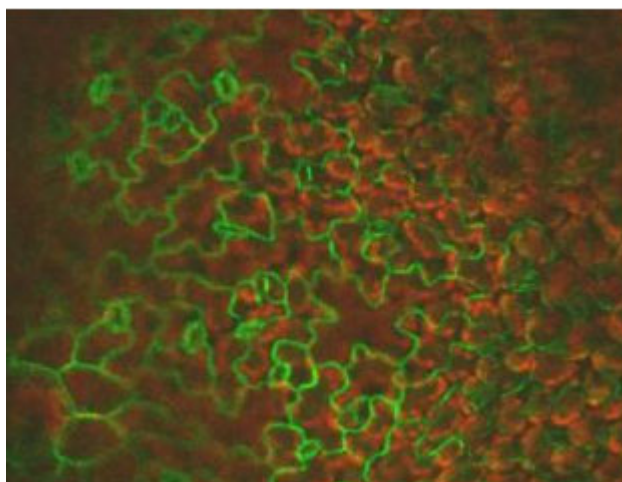


Figure 2. The PEN1-syntaxin is located at the margin of Arabidopsis leaf epidermal cells, demonstrated by confocal microscopy of a transgenic line expressing a GFP-PEN1 fusion protein. Together with what otherwise is known about syntaxins, this strongly indicate that the PEN1-protein is plasma-membrane bound.

The importance of vesicle trafficking in penetration resistance has previously been suggested based on transmission electron microscopy of host plants (e.g. Zeyen and Bushnell, Can. J. Bot. (1979) 57, 898-913) and use of inhibitors in non-host plants (e.g. Mellersh et al., Plant J. (2002) 29, 257-268). We are now able to add conclusive evidence based on genetics to this. The fact that we have made this discovery using a non-host situation, confirms the view that the defence components often are similar in host and non-host interactions.

Effect of barley-legume intercrop on disease in an organic farming system

Julia (Síle) Kinane and Michael Lyngkjær

Resistance Biology Programme

In Danish (and European) organic farming, there is an urgent requirement for increased production of protein and cereal crops to meet the increasing demand for feeding monogastric animals (pigs and poultry). Grain legumes and cereals are complementary in animal feeds, legumes providing high levels of protein and cereals supplying carbohydrate. Intercropping (which is defined as growing two or more crops together) allows the simultaneous cultivation of grain legumes and cereals, in mixture which can be harvested together and fed directly to animals as a mixture. The FØJO-funded project (GENESIS) aims to evaluate the potential for increasing protein production by intercropping in an organic cropping system. The Resistance Biology Programme is participating in this project by investigating the effect of intercropping on disease incidence.

Barley-legume intercrops were grown according to organic principles over two years at two different locations in Denmark. Barley was intercropped with lupin, faba bean or pea.

Crops were grown as a monocrop, double intercrop, triple intercrop or quadruple intercrop. In order to investigate if intercropping had any effect on disease incidence, plants were scored for disease throughout the growing season. The effect of added N on disease was also determined.



Double intercrop; barley and pea.



Quadruple intercrop; barley, pea, lupin and bean

Several diseases were observed. On barley, net blotch (*Pyrenophora teres*), barley leaf rust (*Puccinia hordei*) and powdery mildew (*Blumeria graminis* f. sp. *hordei*) (in order of incidence) were detected. The following diseases were also monitored: ascochyta blight (*Mycosphaerella pinodes*) on pea, brown leaf spot (*Pleiochaeta setosa*) on lupin and chocolate spot (*Botrytis fabae*) on bean. Results for net blotch are graphed on Figure 1. Results for all diseases observed are presented on Table 1.

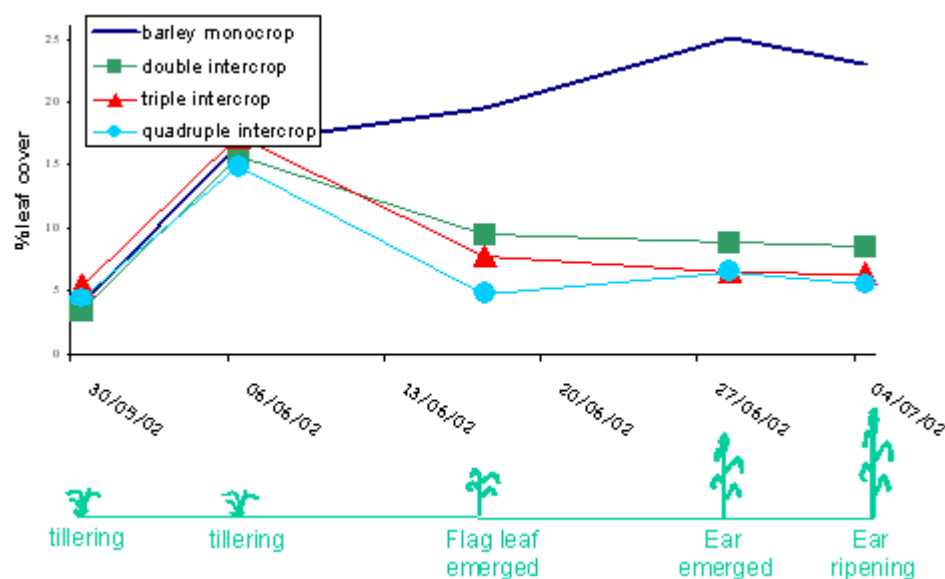


Figure 1: Effect of intercropping barley with grain legumes in double triple, or quadruple intercrop on net blotch incidence. During stem elongation, there was a reduction in net blotch in the intercrops compared to the monocrop. The reduction increased in magnitude with increasing complexity of the intercrop

During stem elongation there was a reduction in net blotch on barley in intercrops. The reduction increased in magnitude with the number of crops in the intercrop (Figure 1). The barley-legume intercrop reduced levels of all diseases observed (Table 1). This reduction was statistically significant for some diseases, but not for others. They differed with respect to their mechanism of dispersal and their response to added N.

Table 1. Effect of barley legume intercrop on all diseases observed in 2002. Dispersal mechanism and response to added N (50 kg urea) are also shown.

Crop / Disease	Effect of intercrop	Dispersal mechanism	Effect of added N
Net blotch (<i>Pyrenophora teres</i>)	46% decrease ($p < 0.05$)	splash	no effect
Brown rust (<i>Puccinia hordei</i>)	22% decrease ($p > 0.05$)	wind	18% increase
Powdery mildew (<i>Blumeria graminis</i>)	21% decrease ($p > 0.05$)	wind	26% increase
PEA Mycosporella spot (<i>Mycosporella pisi</i>)	58% decrease ($p < 0.05$)	splash	no effect
LUPIN Brown spot (<i>Pleiochaeta setosa</i>)	80% decrease ($p < 0.05$)	splash	not tested
BEAN Chocolate spot (<i>Botrytis</i>)	22% decrease ($p > 0.05$)	wind	16% increase

Expert Groups

- Analytical Tools
- Applying and monitoring environmental stress
- Bio-imaging and Micromanipulation
- Bioinformatics

- [Plant Transformation and Reverse Genetic Tools](#)

Analytical Tools expert group

Objectives

The Analytical Tools - expert group in the Plant Research Department shall ensure the maximum exploitation of equipment for proteomics, metabolomics, isotope studies and general analytical chemistry. The expert group aims to increase the visibility of the tools available and their potential application and thereby stimulate interaction between the research programmes.

The Plant Research Department operates a wide range of advanced analytical instruments in order to meet the research taking place at all levels from genetics to applied chemistry. However, the instrumentation naturally relates to a number of analytical disciplines. The Department meets well the state-of-the-art within separation science. Thus, in 2002 a new automated capillary electrophoresis system incl. dual wave length laser induced fluorescence (Beckman) has been installed, two nano-LC systems (LC-Packings) have been interfaced to a LCQ ion trap mass spectrometer and a Q-ToF UltimaGlobal mass spectrometer, respectively. The latter instrument was likewise acquired in 2002. In addition, the Department operates a large number of GC and LC systems with a broad pallet of detection systems. The research strongly benefits from the use of stable as well as radioactive isotopes and scintillation counters, radio imaging systems and isotope ratio mass spectrometers are available. Finally, it must be mentioned that our genetic studies to a great extent involve the use of DNA sequencing strategies as well as PCR instruments.

Available technologies in the Plant Research Department – an overview

Instrumentation	Units	Main use
Liquid chromatography (LC)	5	Metabolites/plant products/pollutants
Capillary electrophoresis (CE)	1	Metabolites/proteins and peptides
Liquid chromatography/mass spectrometry (LC/MS/MS)	2	Peptides/metabolites/polymers
Gas chromatography (GC)	3	Metabolites/environmental chemistry
Gas chromatography/mass spectrometry (GC/MS)	1	Bio-energy/metabolites
Gas chromatography/mass spectrometry (GC/MS/MS)	2	Pollutants/metabolites
Mass spectrometers (MS)	4	Isotope ratios/energy research
Spectrometers (UV/VIS/IR)	3	Metabolites/proteins/gaseous products
Analytical flash pyrolyzers	2	Biomass/biopolymers
Scintillation and nuclear imaging systems	2	Tracer studies

Technologies established in 2002

- [High performance liquid chromatography on the nano-scale](#)
- [Q-ToF Ultima Global](#)

Projects bridging the research programmes

- Plant mitochondria (PLP and PLN)
- The functional proteomics of mitochondria from rice (*Oryza sativa*) (PLP and PLN)
- Mass spectrometric analysis of the uptake and metabolism of ammonium in mycorrhiza (PLN and PLE)
- Mass spectrometric analysis of nucleotide sugars (PLN and PLP)
- Proteomics of the peribacteroid membrane in legumes (PLR and PLN)
- Proteomics of the plant-powdery mildew interaction (PLR and PLN)

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Applying and monitoring environmental stress**Objectives:**

To provide the Plant Research Department with tools, expertise and resources including

- Further development of growth facilities to study the effects of increased temperature, carbon dioxide and ozone
- Ecophysiological methods for measurements of abiotic and biotic stress
- Mathematical analyses of dynamics of crop systems (e.g. plant growth, plant competition and epidemiology)
- Developing *Arabidopsis* as a crop model (parallel field experiments and experiments under controlled conditions (RERAF))
- *Arabidopsis* as a model for crop plant disease resistance in collaboration with expert group on *Bioinformatics and Genetics*
- Seed quality (important for cereals and oil crops) and other quality traits (fibres)
- N/C distribution

Available technologies**Growth facilities**

- *Greenhouse #23*: App. 100 m² (ground) distributed at 34 compartments ranging from 1 to 9 m². Classified to class 1 for work with transgenic plants
- *Greenhouse #24*: App. 130 m² (tables) in two compartments. Plan for classification to class "plants" for work with transgenic plants.
- *Greenhouse #25*: 3 App. 50 m² (tables) and 160 m² (ground) in three compartments. Classified to class "plants" for work with transgenic plants.
- *Growth chambers Building 318*: App. 45m² in 14 chambers ranging from 0.8m² to 10 m². Some chambers are classified to class 1 for work with transgenic plants.
- *Growth chambers Building 300*: App. 10.6m², 4 chambers all 2.9m².
- *Growth chambers Building 301*: App. 19 m², 2 chambers 13.5 & 5.4m².
- *Growth chambers Building 330*: 18 m², 2 chambers both 9m².
- *Growth chambers RERAF, Building 325*: 176 m², 10 chambers ranging from 7 to 14 m². Two chambers classified to class 1 for work with transgenic plants. Two chambers with enhanced CO₂ levels.

Ecophysiological tools

Gas exchange equipment (CO₂ and/or H₂O):

- CIRAS-1 with automatic leaf cuvette. (Leaf level: Net photosynthesis, stomatal conductance and transpiration -temperature, light and CO₂ response curves)
- Li-Cor, LI-6400 with soil and leaf chambers. (Soil respiration and leaf level: Net photosynthesis, stomatal conductance and transpiration -temperature, light and CO₂ response curves)
- ADC LCA3 ((Net photosynthesis, stomatal conductance and transpiration)
- Li-Cor, LI-1600 steady state porometer. (stomatal conductance)

Optical equipment:

- Handy PEA (Chlorophyll fluorescence kinetics)
- SPAD 502 (relative chlorophyll content)
- CropScan MSR87 (multi spectral radiometer)
- Ocean optics SD2000 (miniature fibre-optic spectrometer)

Ongoing projects across programs

Arabidopsis as a crop model.

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Bio-imaging and Micromanipulation

Objectives:

In addition to insure cross departmental use of equipment at Risø for bio-imaging and micromanipulation this expert group also aims at supporting development of new research area by sustaining relevant expertise and hardware

- Establish microscope facility (light, fluorescence and confocal) including micro-manipulation, injection and extraction
- Single cell gene expression analyses and manipulations
- Localization of transcripts, proteins and metabolites in time and space
 - *In vivo (situ)* detection methods
 - *In vivo/vitro* NMR
 - *In vivo* optical methods
 - Radiography
 - *In situ* RNA hybridisation
 - Developing nano-probes and use commercially available probes
- Electron microscopy on biological material (MRD EM facility)

Ongoing projects:

NMR equipment at Risø and RUC is available for both *in vitro* and *in vivo* studies of metabolites and metabolic pathways. Fluorescent nano-probes suitable for plant cell studies will be developed in collaboration with POL. The potential use of optical coherence tomography for imaging in plant research is being investigated with OFD. Our phosphorimager is an excellent tool for time course studies of assimilation and transport of radio-labelled compounds. A scintillation counter is available for the required quantification of radioisotopes in the *in vivo* studies. Detecting expression of green fluorescent protein (GFP) gene fusions in plant tissue using the confocal microscopy facilities in POL.

A list of equipment is available at http://www.risoe.dk/pbk/research_uk/plp/equipment.htm

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Bioinformatics

Objectives:

- To organise events where experts get together across programmes within the areas
 - Bioinformatics (sequence analysis)
 - Mathematical modelling
 - Statistics
 - Databases (e.g. Seed Database)
 - Theoretical genetics (Gene mapping, QTL-analysis, population genetics)
- To establish a forum to be contacted by non-experts to get inspiration and help on tools and services in relation to Data management and mining.
- To keep track of software in the department related to these areas and to update this information. The list of software is available at http://www.risoe.dk/pbk/research_uk/plr/software.htm.

Activities organised in 2002:

- Several meetings with different groups of scientists being potential users of the Risø Seed Database under reconstruction.
- Presentation of examples of data mining and management tools and problems in PRD
- Discussion on Sequence analyses

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Plant Transformation and Reverse Genetic Tools

Objectives:

To provide the Plant Research Department with tools, expertise and resources including

- Transformation platform for *Arabidopsis*, Barley and *Brachypodium*
- Delivery of reverse genetic tools
 - Antisense
 - T-DNA insertions
 - Transposon mutagenesis
 - RNAi
 - Transient expression system for tissue specific studies or interaction with microbes

The aim is to provide key techniques and mutagenised populations to PRD.

We emphasise especially the development of *Brachypodium distachyon* as a test bed for monocot-specific issues.

Brachypodium belongs to the Pooideae subfamily as many of the agronomical important crops. It exhibits unique monocot model plant characteristics having the smallest known genome size in grasses of down to 123 Mbp/1C, a life cycle of less than four months and a physical size of down to 20 cm at maturity. It is a self-fertile inbreeding annual with a chromosome base number of five. Di-, tetra- and hexaploid cytological genotypes have been collected mainly from South/East Europe. Many of the diploid accessions require vernalisation in order to flower (in contrast to tetra- and hexaploid genotypes). To make *Brachypodium* an efficient alternative model plant for functional genomics in cereals and grasses, an easy, fast, and efficient transformation system is currently developed with stable transformation rates up to 25%. At the same time other key techniques (e.g. chemical mutagenesis) are developed or established.

Overview of available reverse genetic tools at PRD

Technique/Item	<i>Arabidopsis</i>	Barley	<i>Brachypodium</i>	Remarks
Overexpression	✓	✓	✓	Several vectors available
Antisense/RNAi	✓	(✓) soon available	(✓) soon available	Several vectors available
Chemical mutagenesis (technique)	✓	✓	✓	
Chemical mutagenesis (population)	-	✓	In preparation	
T-DNA insertion mutagenesis (technique)	✓	-	-	
T-DNA insertion mutagenesis (population)	✓	-	-	
TILLING	-	-	-	To be established
Transient expression system	-	✓	✓	
Stable transformation (biolistic)	-	✓	✓	
Stable transformation (<i>Agrobacterium</i>)	✓	✓	✓	
Selection systems	Bar, Hyg, Kan	Bar	Bar, Hyg	

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Bykova, Natalia (until 31.08.02)
Christiansen, Solveig Krogh
Didion, Thomas
Egsgaard, Helge
Engvild, Kjeld C.
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Gissel Nielsen, Gunnar (until 28.02.02)
Gjetting, Torben
Gundersen, Vagn (until 30.09.02)
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Hauser, Thure
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Olsen, Anne
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Tung, Tran Duc Tuan (until 31.05.02)
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Visiting scientists

Araja, Ilona, Institute of Biology, University of Latvia (9 months)
Brazauskas, Gintaras, Lithuanian Institute of Agriculture (2 months)
Eriksen, Lars B., Sejet Plant Breeding, Denmark (1 year)
Fritz, Maendy, University of Giessen, Germany (5 months)
Hindsgaul, Claus, Danish Technical University, Lyngby, Denmark (3 months)
Igamberdiev, Abir, Umeå University, Sweden (8 months)
Lababidi, Samer, International Center for Agricultural Research (ICARDA), Aleppo, Syria (3 months)
Lang, Sara, Institute of Biotechnology, University of Helsinki, Finland (3 weeks)
Medina, Carlos Martin, Department of Chemistry and Chemical Engineering, University of Matanzas, Cuba (2 months)
Meier, Matthias, Geobotanical Institute, Swiss Federal Institute of Technology, Zürich, Switzerland (2 months)
Offen, Nils, University of Bayreuth, Germany (6 months)
Orabi, Jihad, International Center for Agricultural Research (ICARDA), Aleppo, Syria (5 months)
Smith, Andrew, Faculty of Science, University of Adelaide, Australia (5 months)
Smith, Sally, Department of Soil Science, University of Adelaide, Australia (5 months)
Stewart, Keith, Department of Plant Sciences, University of Oxford, UK (3 weeks)
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Articles in international journals, books and reports

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Acronyms

ACT	Agmatine coumaroyltransferase	NTP	Nucleoside triphosphate
AM	Arbuscular mycorrhizal	OECD	Organisation for European Economic Co-operation
ANOVA	Analysis of Variance	OTA	Ochratoxin A
cDNA	complementary DNA	Q-TOF	Quadrupole-time of flight
GFP	Green Fluorescent Protein	P	Phosphate
GUS	Beta-glucuronidase (Beta-D-glucuronoside glucuronosohydrolase)	PEN1	gene required for penetration resistance
HCBT	Anthranilate N-hydroxycinnamoyl/benzoyltransferase	PHT	Putrescine N-hydroxycinnamoyltransferase
IEF	Isoelectric focusing	P _i	Inorganic phosphate
K _d	Distribution coefficient	PolyP	Polyphosphate
K _F	Adsorption constant	RERAF	Risø Environmental Risk Assessment Facility
KVL	The Royal Veterinary and Agricultural University (Den Kgl. Veterinær- og Landbohøjskole)	RVAU	= KVL
LC	Liquid chromatography	THT	Tyramine N-hydroxycinnamoyltransferase
LCQ	Liquid chromatography quadrupole-ion storage	UidA	= GUS
MS	Mass spectrometry	VP16	Viron Protein 16
NMR	Nuclear magnetic resonance	ZON	Zearalenone

Bibliographic Data Sheet

Risø-R-1398 (EN)

Title and authors

Plant Research Department Annual Report 2002

J. Kossmann, I. Jakobsen, K.K. Nielsen, K. Pilegaard, S.K. Rasmussen, H. Thordal-Christensen

ISBN 87-550-3194-3(Internet)

ISSN 0106-2840

ISSN 1602-0103

Pages html documents: 22

Date: April 2003